201-14517

NCIC HPV
Sent by: Mary-Beth Weaver

06/03/2003 11:17 AM

To: NCIC HPV, moran.matthew@epa.gov
cc:
cc:
Subject: Public comments on the Ferro HPV test plan for 2-ethylhexyl diphenyl phosphate

Jessica Sandler <jessicas@peta.org> on 05/29/2003 12:35:37 PM

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cc: Priscilla Flattery/DC/USEPA/US@EPA, Oscar Hernandez/DC/USEPA/US@EPA, Stephen Johnson/DC/USEPA/US@EPA

Subject: Public comments on the Ferro HPV test plan for 2-ethylhexyl diphenyl phosphate

Dear Mr. Johnson,

Attached please find the comments of the American animal protection community on Ferro Corporation's HPV test plan for 2-ethylhexyl diphenyl phosphate. I am calling your attention to it as it, along with the Ferro plan we submitted comments on yesterday, is particularly egregious. We are asking that you review our comments prior to issuing your own and that you address the fact that existing data have been ignored by the company while proposing to kill a large number of animals as well as the other concerns detailed in our comments.

Thank you,

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HPV test plan comments -- (Ferro) 2EHDPP.doc

201-14517

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May 29, 2003

Christine Todd Whitman, Administrator
US Environmental Protection Agency
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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Subject: Comments on the HPV test plan for 2-ethylhexyl diphenyl phosphate

Dear Administrator Whitman,

The following are comments on the HPV program test plan for 2-ethylhexyl diphenyl phosphate (EHDPP; CAS no. 1241-94-7), submitted by Ferro Corporation. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal protection, health, and environmental organizations have a combined membership of more than ten million Americans.

This test plan violates both the October 1999 agreement to reduce the number of animals killed in the HPV program and the original HPV framework agreement to which all participants subscribed, in that it ignores existing data while proposing to poison more than 750 mammals and 40-120 fish. Ferro is proposing to conduct an acute fish toxicity test (OECD no. 203), a mammalian combined repeat-dose, reproductive and developmental toxicity test (OECD no. 422), and a mammalian erythrocyte micronucleus test (OECD no. 474). Yet judging from the large amount of data that we found simply by a cursory examination of several databases – with very little effort – it appears that Ferro was unwilling to spend the time and effort necessary to prepare a satisfactory test plan.

It is egregious that Appendix 1 of the test plan (the "summaries") refers to only two previous toxicity studies (acute and repeat-dose oral toxicity studies in rats), yet numerous studies on the toxicity of EHDPP have been carried out. The data from several studies have been published, as detailed below. In addition, the data from at least 42 corporate studies have been submitted to the EPA. The EPA submissions, listed at the beginning of the references, are available to Ferro under the Freedom of Information Act, and the EPA clearly has access to them. The test plan provides no explanation as to why Ferro has disregarded almost all available data. Indeed, it does not explain why Ferro considers one of the studies to which it refers in its own Appendix, the repeat-dose toxicity test, to be unreliable (p. 2).

A second deficiency is that the test plan provides little information about the use of and human exposure to EHDPP. The test plan states merely that EHDPP is a general-purpose plasticizer for most commercial resins, and does not mention its other applications, which include use as an additive in lubricating oils and hydraulic fluids (Boethling 1985). The test plan states vaguely that EHDPP is approved for indirect food contact (p. 2), referring to its approval by the FDA for use as a component (a

plasticizer) of adhesives or coatings in contact with food (FDA 2002), but it makes no mention of the large volume of research that has been carried out on its prevalence in foods (Daft 1982, Giam 1987, Castle 1988, Gunderson 1995a, 1995b, KAN-DO Office 1995), nor of the data that supported the FDA's approval.

The published toxicity data include the following:

(i) *Acute oral toxicity in rats.* No toxicity was seen with single doses of up to 4.0 grams per kilogram of body weight (g/kg; Mallette 1952), or up to 24.0 g/kg, which were the maximum doses administered (Treon 1953).

(ii) *Acute oral toxicity in rabbits*. The minimum LD_{50} value was 24.0 g/kg. No no-observed-effect level was determined, and toxic effects seen at lower doses were dose-dependent, and included weight loss and diarrhea (Treon 1953).

(iii) *Subchronic oral toxicity in rats*. Female rats were administered EHDPP for 10 days at 0.3-3.0 g/kg/day. Reduced weight gain and toxic effects were seen at 1.0 g/kg/day and higher (Robinson 1983, 1986). In an earlier study, severe toxicity was not seen with administration for 12 days at up to 10 g/kg/day, although oily feces did occur at lower doses (Treon 1953). In a later study, slight decreases in food intake and body weight gain were found with rats administered approximately 1.8 g/kg/day, and some of these rats had soiling of the fur with urine, and red material around the mouth and eyes. The lowest dose at which any effect has been seen in any study is approximately 0.2 g/kg/day, at which dose increased liver and adrenal gland weights were seen (Noda 1983). These findings are compatible with the study referred to by Ferro in its Appendix, in which toxic effects occurred at 5.0 g/kg/day and above.

(iv) *Subchronic oral toxicity in rabbits*. Severe toxicity was not seen with doses of up to 8.7 g/kg/day, but there was an increased frequency of oily feces in the 2.2-8.7 g/kg/day dose range (Treon 1953).

(v) *Chronic oral toxicity in rats.* With administration in feed for 2 years, the no-observed-effect level was a feed content of 0.125%. A 1.0% feed content resulted in decreased body weight gain and increased death rate (Treon 1953).

(vi) *Chronic oral toxicity in dogs.* With administration in feed for 2 years, the no-observed-effect level was 0.5 mL/kg/day (approximately 0.5 g/kg/day). With 1.0 mL/kg/day (approximately 1.0 g/kg/day), the body weight gain was decreased but no other toxicity was seen (Treon 1953).

(vii) *Acute intravenous toxicity in rabbits*. The minimum LD_{50} value was approximately 0.218 g/kg. The only toxic effect seen at lower doses was weight loss (Treon 1953).

(viii) *Acute intraperitoneal toxicity in rats*. The minimum LD_{50} value was 2.4 g (approximately 18 g/kg). Moderate toxicity occurred at lower doses (Mallette 1952).

(ix) *Dermal toxicity in rabbits*. In the Draize test, no local or systemic effects were found with up to 9.4 mL/kg (approximately 9.4 g/kg) undiluted EHDPP applied to either intact or abraded skin. With administration of 5 mL at hourly intervals for 7 hours, no toxicity was seen with total doses of up to 13.7 g/kg (Treon 1953). In another study, however, EHDPP was found to be a moderate dermal irritant, and to have a moderate sensitizing effect (Mallette 1952).

(x) *Dermal toxicity in humans*. EHDPP had a moderate sensitizing effect (Mallette 1952).

(xi) *Female reproductive toxicity in rats.* No reproductive parameters, including the weights of sexual organs, were affected by oral doses of up to 3.0 g/kg/day (Noda 1983, Robinson 1983, 1986).

(xii) *Developmental toxicity in rats.* The conclusion was that there was no developmental toxicity at oral doses of up to 3.0 g/kg/day (Noda 1983, Robinson 1983, 1986).

(xiii) *Embryotoxicity and developmental toxicity in domestic fowl*. A single injection of 0.05 mL (approximately 0.85 g/kg) into the yolk reduced the hatch rate from 90.1% to 80%. There were also some (unstated) effects on the chicks after hatching (McLaughlin 1962). The no-observed-effect level was later determined to be 1.0 g/kg (McLaughlin 1965).

(xiv) *Ames test.* No mutagenicity was shown with four *Salmonella typhimurium* strains at 100-10,000 ì g/plate (Chase 1983, Zeiger 1985).

The incompleteness of the test plan makes it difficult to critique. We urge the EPA to require the preparation and resubmission of a complete test plan. Therefore, the following criticisms of the test plan as it currently stands are merely provisional:

1. Mammalian combined repeat-dose, reproductive and developmental toxicity test

Clearly, animal data are already available, and Ferro displays a criminal failure in its neglect of these data, opting instead to simply poison more animals. The published data show that EHDPP has moderate chronic and subchronic toxicity in dogs, rats and rabbits. On the other hand, no mammalian developmental or reproductive toxicity was found, even at doses that resulted in marked maternal toxicity.

The animal data suggest that reproductive and developmental toxicity due to EHDPP is unlikely at doses that do not give rise to chronic and subchronic toxicity. However, these data are unlikely to be directly applicable to humans, because there are major interspecies differences in the developmental toxicity of compounds of this type ("The rat embryo seems to be less susceptible to OP [organophosphorus] compounds than the mouse embryo"; Kitos 1992, p. 396), so further animal data are unlikely to be of much value. Exposure and epidemiology studies are therefore appropriate. If the data obtained suggest that there is cause for concern, then, assuming that the aim is to reduce real-world

hazards rather than to obtain theoretical data, priority should be given to technical and legislative approaches to exposure reduction, rather than to additional animal data generation.

Finally, an *in vitro* method for screening for developmental toxicity is available (see Appendix).

2. *In* vivo genetic toxicity test

Again, existing data have been ignored. In addition, under the HPV program, the EPA has issued an official statement, in the *Federal Register*, that *in vivo* genotoxicity tests should only be used if "known chemical properties" preclude the use of an *in vitro* test:

> *Persons who conduct testing for chromosomal damage are encouraged to use in vitro genetic toxicity testing (Mammalian Chromosomal Aberration Test) to generate needed genetic toxicity screening data, unless known chemical properties preclude its use. These could include, for example, physical properties or chemical class characteristics. With regard to such cases, test sponsors are asked to submit to EPA the rationale for conducting one of these alternative tests … as part of the test plan. (EPA Federal Register 2000, p. 81695)*

Ferro has provided no explanation for its decision to use an *in vivo* method and must justify doing so based on chemical properties precluding its use or use an *in vitro* method.

3. Acute fish test

(a) *The partition coefficient of EHDPP is too high*. Ferro proposes determining the partition coefficient (p. 3). However, the log $K_{o/w}$ value is already known to be 5.73 (Saeger 1979), and the EPA has clearly stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2. The EPA recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of acute fish and *Daphnia* tests (EPA *Federal Register*, December 2000, p. 81695).

(b) *The ecologic significance of fish tests should be taken into consideration.* Ecotoxicity and mammalian toxicity tests have different purposes: mammalian tests are assumed to be useful for predicting toxicity in individual humans, whereas fish tests are not for predicting toxicity in individual fish, but for predicting economic loss (to commercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The fish test therefore aims to show whether exposure to EHDPP will result in large-scale fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. However, the toxicity of EHDPP towards

these types of organism is unknown, as shown by the inclusion in the test plan of tests on aquatic invertebrates and algae (p. 3). Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

(c) *Several* in vitro *and* in silico *alternatives are available*. See Appendix.

In this context, we must reiterate a number of points made by the EPA in its October 1999 letter to HPV program participants (EPA 1999):

> 1. In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested.
>
> 2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing.
>
> 5. Participants are encourage to use *in vitro* genetic toxicity data to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.
>
> 8. … As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant.

Ferro's test plan is not only a blatant violation of the above-mentioned October 1999 letter, but of the original HPV framework agreement to review and submit existing data. Once again, we urge the EPA to reject this plan and to require the preparation and resubmission of a satisfactorily researched test plan.

Thank you for your attention to these comments. We can be reached via e-mail at RichardT@PETA.org.

Sincerely,

Jessica Sandler, MHS
Federal Agency Liaison
People for the Ethical Treatment of Animals

Richard Thornhill, PhD
Research Associate
PETA Research and Education Foundation

Appendix: *In vitro* and *in silico* test methods

1. In silico *fish test substitute*. Quantitative structure activity relationship (QSAR) programs provide *in silico* methods for estimating toxicity to fish and other aquatic organisms. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

2. In vitro *fish test substitutes*:

   (i) TETRATOX is an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997). With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997), and the extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

   (ii) The test protocol and performance parameters of the recently validated *Dar*T test are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, it uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

3. *Mammalian developmental toxicity test substitute*. An *in vitro* embryotoxicity test method, the rodent embryonic stem cell test, has recently been validated by the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). This test is now commercially available in the U.S. We therefore urge Ferro to consider the use of this *in vitro* test. If a positive result is found in the embryonic stem cell test, EHDPP should be treated as a developmental toxicant/teratogen, and no further testing should then be carried out within the screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than six months, we have received no reply. We urge Ferro to correspond directly with the EPA on the incorporation of this validated non-animal test.

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